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Draft NTP Technical Report TR 577 on a Nondecolorized Whole Leaf Extract of *Aloe barbadensis* Miller (Aloe vera)

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NTP Technical Reports Peer Review Meeting

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Background

- Herbal remedies
  - Plants or plant parts, and extracts or mixtures of these that are used to prevent, alleviate, or cure disease
  - Approximately 38% of the U.S. adult population use herbal products
  - Use based primarily on historical or anecdotal information
- Aloe vera
  - Synonym for the *Aloe barbadensis* Miller plant
  - Centuries old history of lay acceptance as an herbal remedy
  - Used traditionally for cosmetic applications, dietary supplementation, prophylaxis therapy, and medicinal treatment of a broad spectrum of illnesses
  - Products utilize various Aloe vera leaf components in their formulations

Study Rationale

- Aloe vera was nominated by the National Cancer Institute to the NTP for dermal and oral toxicity and carcinogenesis testing
  - Aloe vera is a widely used cosmetic, dietary supplement, and herbal remedy
  - Potential for widespread dermal and oral human exposure
  - Components in Aloe vera may possess tumor-promoting activities
  - Lack of toxicity information
  - Dermal exposure studies conducted previously (NTP TR-553)
  - Present study examined exposure by the oral route

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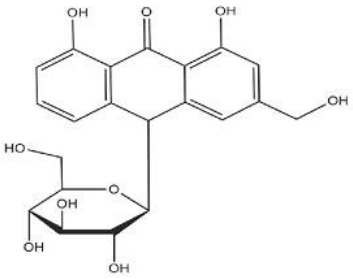
Test Material Characterization and Dosed Solutions Certification

- Test material
  - Obtained from fresh *Aloe barbadensis* Miller plants
  - Lyophilized (6% moisture) within 6 h of harvest and γ-irradiated
  - Compositional analysis (Covance)
  - Homogeneity analysis (malic acid, aloin A, and aloe-emodin)
  - Molecular weight
  - Stability
  - Glycosyl linkage analysis
- Dosed water solutions
  - Homogeneity
  - Stability at room temperature and 2 – 8 °C
  - Dose certifications (weekly monitoring of malic acid, aloin A)

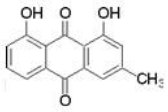
Aloe Vera Components Monitored Throughout Studies

- Aloe-emodin
  - Bulk material monitoring
- Aloin A and malic acid
  - Bulk material monitoring
  - Dosed water solutions monitoring weekly
- Carbohydrates
  - Bulk material testing
  - Molecular weight
  - Glycosyl linkage analysis

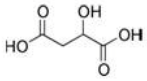
Aloin A



Aloe-emodin



Malic acid



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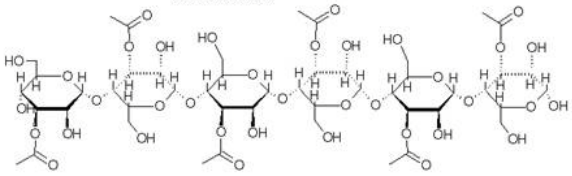

Aloe Vera 14-Day Range-finding and Metabolism Study

- Male and female F344/N rats and B6C3F$_1$ mice; 4 animals/sex/treatment
- Test materials
  - Aloe vera gel, Aloe vera decolorized whole leaf, and Aloe vera nondecolorized whole leaf plant extracts
- Doses
  - 0, 0.5, 1, 1.5, 2, and 3% (wt/wt) in drinking water for 14 days
  - Formulation prepared and water bottles issued daily
- Parameters measured
  - Water and feed consumption and body weights
  - Hematology and clinical chemistry
  - 24 h urine chemistry and G.I. transit time (4 wk intervals)

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Aloe Vera Nondecolorized Whole Leaf 13-Week Toxicity and Metabolism Study

- Male and female F344/N rats and B6C3F1 mice; 12 animals/sex/treatment
- Doses
  - Toxicity study (0, 1, 2, and 3% wt/wt)
  - Metabolism study (0 and 2% wt/wt, rats); (0 and 3% wt/wt, mice)
  - Formulations prepared 3x/week and water bottles issued daily
- Parameters measured
    - Water and feed consumption and body weights
  - Metabolism study
    - 24 h urinalysis and G.I. transit time (4 wk intervals)
    - Hematology and clinical chemistry

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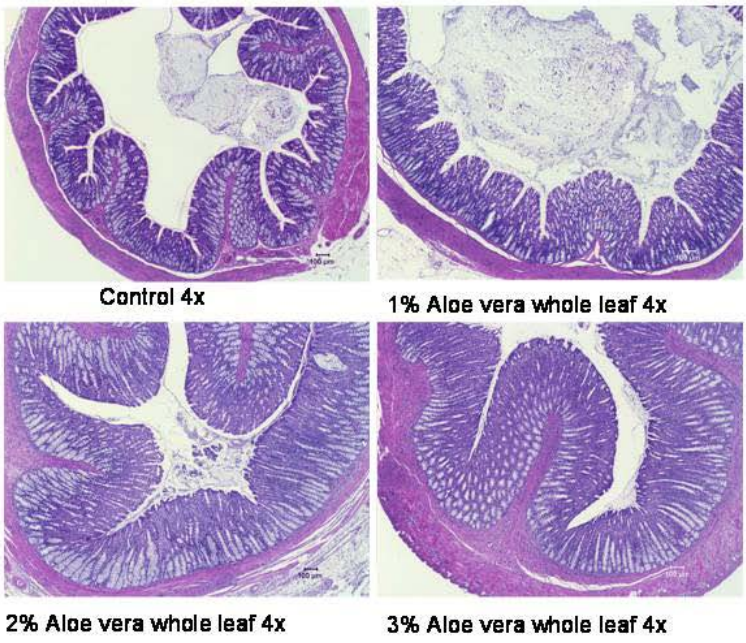
Goblet Cell Hyperplasia in F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 13-Weeks



Control 4x

1% Aloe vera whole leaf 4x

2% Aloe vera whole leaf 4x

3% Aloe vera whole leaf 4x

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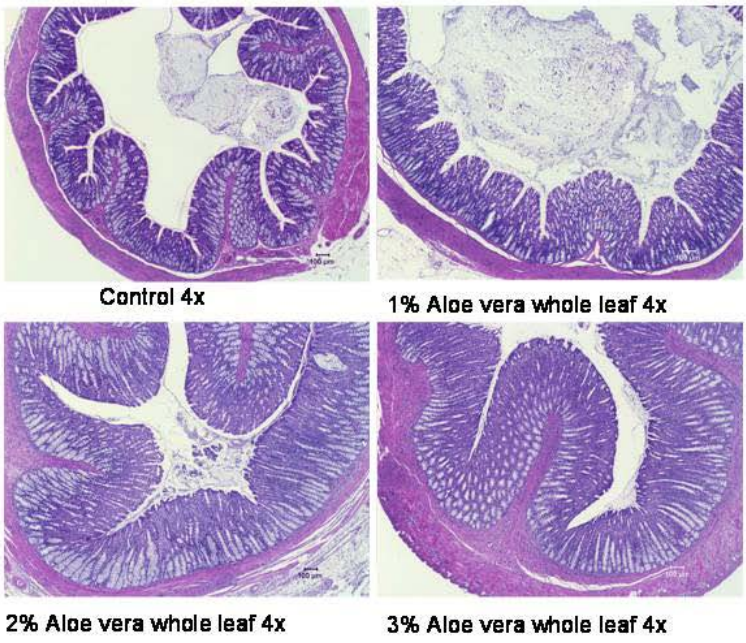

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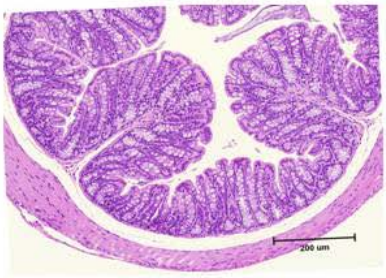
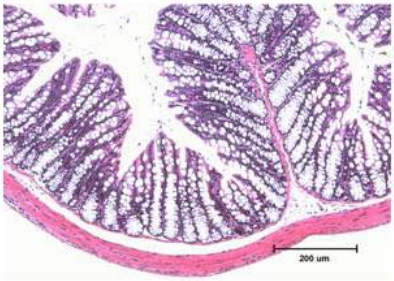
Goblet Cell Hyperplasia in B6C3F1 Mice Administered Aloe Vera Nondecolorized Whole Leaf Extract for 13-Weeks

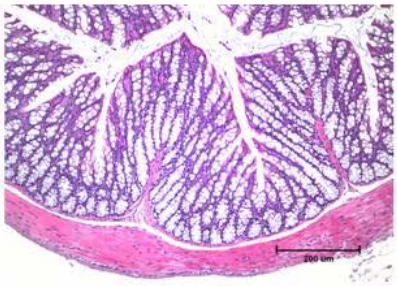


Control 10x

1% Aloe vera whole leaf 10x



2% Aloe vera whole leaf 10x



3% Aloe vera whole leaf 10x

2-year Study on Aloe Vera Nondecolorized Whole Leaf Extract

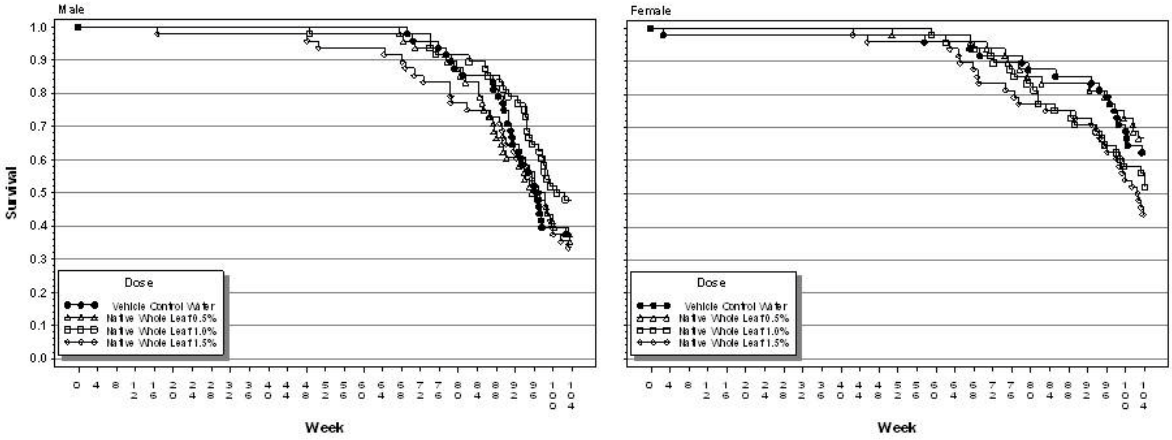
- Male and female F344/N rats and B6C3F1 mice
  - 48 animals/sex/treatment
  - Concentrations: 0, 0.5, 1, and 1.5% (wt/wt water) for rats
  - Concentrations: 0, 1, 2, and 3% (wt/wt water) for mice
- Drinking water continuous 104 weeks
  - Formulation prepared 3x/week
  - Water bottles issued Mon, Wed, Fri, and Sun

Chemical Characterization of Aloe Vera Nondecolorized Whole Leaf Extract and Dosing Solutions

- Bulk test material
  - Homogeneity analysis: malic acid 186-203 mg/g; aloin A 6-7 mg/g; aloe emodin 70.5 μg/g
  - Molecular weight: 52 – 78 Kda
  - Glycosyl linkage analysis: prominent 4 linked mannopyranose residues
  - Stability: unchanged throughout study
- Dosing solutions
  - Concentrations of 0, 0.5, 1, 1.5, 2, 3% (wt/wt) in drinking water
    - Malic acid contents 0.98, 1.95, 2.92, 3.64, and 5.84 mg/g water
    - Aloin A contents 32, 66, 98, 131, 197 μg/g water
  - Stability assessed for 96 h (>80% initial at 48 h)

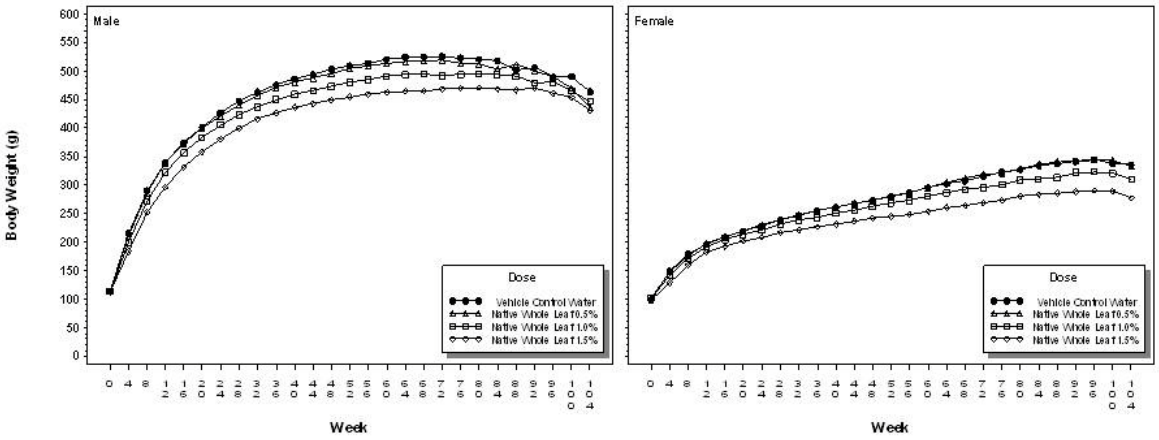
Survival of F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

- Survival of male rats similar to controls; female rats showed decreased survival compared to controls



Body Weights of F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

- Dose-related decreasing trends in body weights in males and females
  - Mean body weights over the study were approximately 90% and 85% of controls for 1.5% Aloe whole leaf groups of male and female rats, respectively



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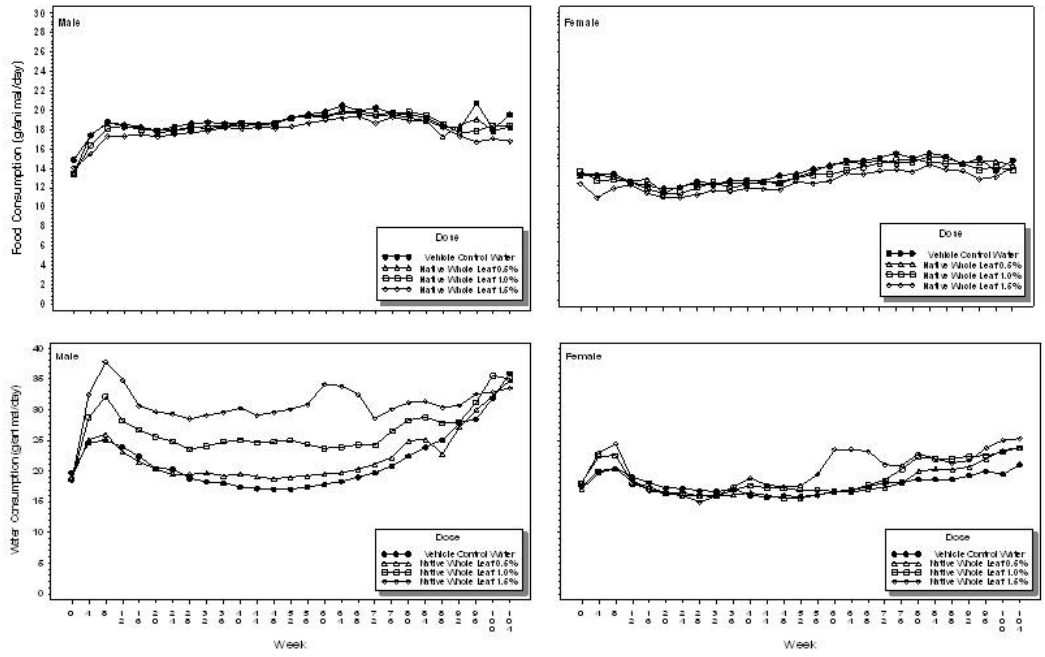
Feed and Water Consumption of F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years



Neoplasms in the Large Intestine of F344/N Male Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years (Clear evidence)

	Aloe vera Nondecolorized Whole Leaf Extract (wt/wt)			
	0%	0.5%	1.0%	1.5%
Adenomas	0/47[a] (0%)***	0/48[b] (0%)	26/48[b] (54%)***	23/48[b] (48%)***
Carcinomas	0/47 (0%)***	0/48 (0%)	10/48 (21%)***	14/48 (29%)***
Adenomas or Carcinomas	0/47 (0%)***	0/48 (0%)	28/48 (58%)***	31/48 (65%)***
Historical Controls (Large Intestine Adenomas or Carcinomas)	0/623			

[a] Asterisks under the 0% control incidence are associated with linear dose trend tests.

[b] Asterisks under dose group incidence are associated with pairwise comparisons to control

*** Significant ($p<0.001$)

Neoplasms in the Large Intestine of F344/N Female Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years (Clear evidence)

	Aloe vera Nondecolorized Whole Leaf Extract (wt/wt)			
	0%	0.5%	1.0%	1.5%
Adenomas	0/48[a] (0%)***	0/48[b] (0%)	6/48[b] (13%)*	13/48[b] (27%)***
Carcinomas	0/48 (0%)**	0/48 (0%)	3/48 (6%)	4/48 (8%)*
Adenomas or Carcinomas	0/48 (0%)***	0/48 (0%)	8/48 (17%)**	15/48 (31%)***
Historical Controls (Large Intestine Adenomas or Carcinomas)	0/527			

[a] Asterisks under the 0% control incidence are associated with linear dose trend test.

[b] Asterisks under dose group incidence are associated with pairwise comparisons to control

* Significant ($p<0.05$); ** significant ($p<0.01$); *** significant ($p<0.001$)

Neoplasms in the Large Intestine of F344/N Female Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years (Clear evidence)

	Aloe vera Nondecolorized Whole Leaf Extract (wt/wt)			
	0%	0.5%	1.0%	1.5%
Adenomas	0/48[a] (0%)***	0/48[b] (0%)	6/48[b] (13%)*	13/48[b] (27%)***
Carcinomas	0/48 (0%)**	0/48 (0%)	3/48 (6%)	4/48 (8%)*
Adenomas or Carcinomas	0/48 (0%)***	0/48 (0%)	8/48 (17%)**	15/48 (31%)***
Historical Controls (Large Intestine Adenomas or Carcinomas)	0/527			

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[b] Asterisks under dose group incidence are associated with pairwise comparisons to control

* Significant ($p<0.05$); ** significant ($p<0.01$); *** significant ($p<0.001$)

Non-neoplastic Lesions of the Gastro-intestinal Tract in F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

- Mesenteric lymph node hyperplasia and cystic degeneration
  - Characterized by dilated medullary sinuses and large cystic spaces
  - Dose-related increased incidences in male and female rats
- Mucosal hyperplasia
  - Characterized by thickening of the mucosa due to increased length and complexity of mucosal glands, with no cellular atypia and minimal inflammation
  - Dose-related increased incidences in glandular stomach, small intestine, large intestine, and rectum of male and female rats
- Cecal dilitation
  - Partial obstruction of the cecum due to pressure of contents
  - Dose-related increased incidences in male and female rats

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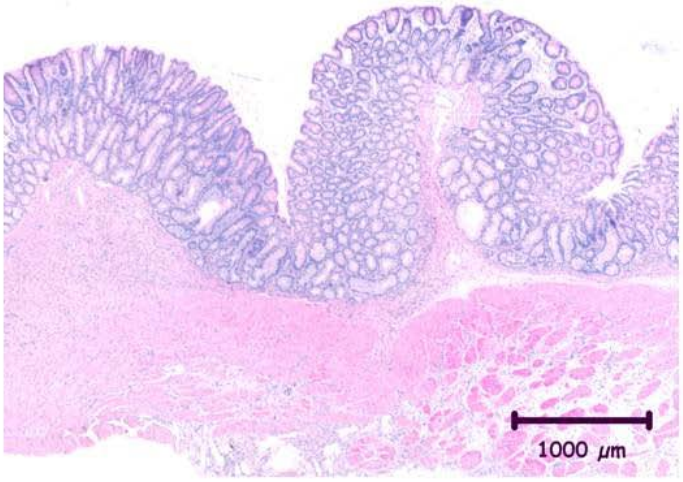
Morphology of Mucosa Hyperplasia of the Gastro-intestinal Tract in F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years



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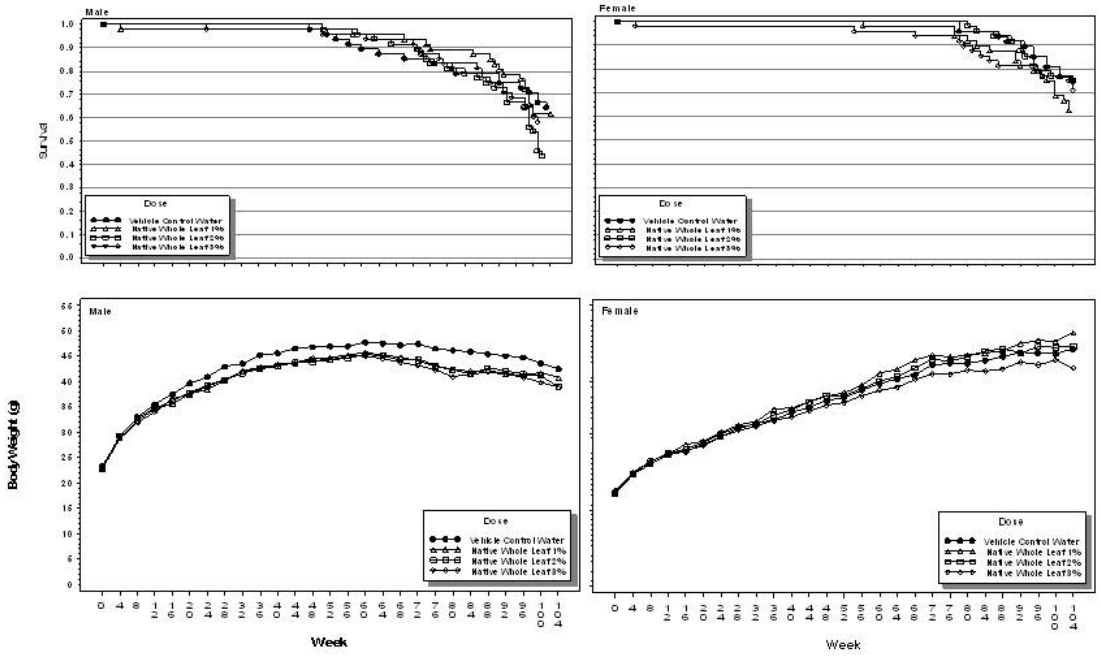
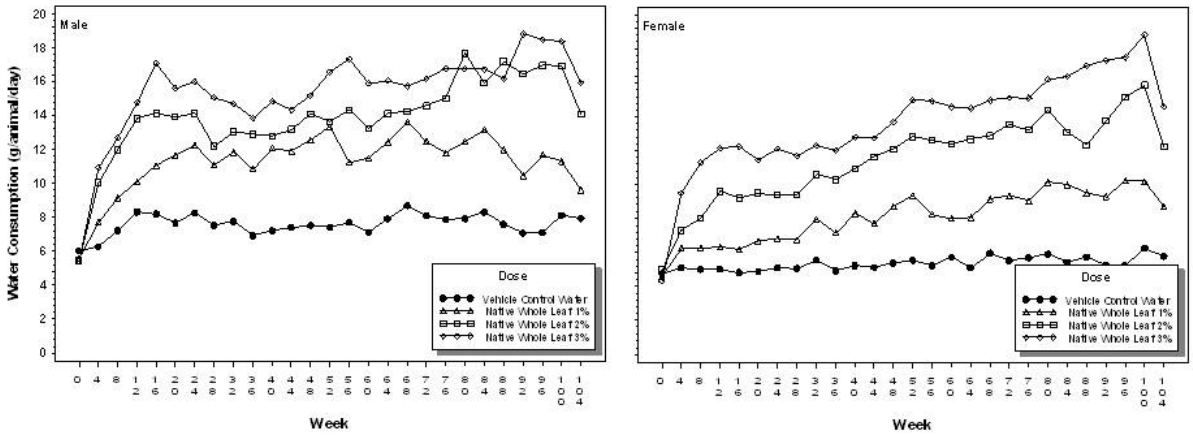
Survival and Body Weights of B6C3F1 Mice Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years



Water Consumption of B6C3F1 Mice Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

- Polydipsia pronounced in male and female mice
  - Water consumption amounts were greater than 200% relative to controls for 3% Aloe whole leaf groups of male and female mice



Non-neoplastic Lesions of the Large Intestine in B6C3F1 Mice Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

	Aloe vera Nondecolorized Whole Leaf Extract (wt/wt)			
	0%[b]	1.0%	2.0%	3.0%
Goblet cell hyperplasia All Sites (Males)	4/47[a] (8.5%)***	17/44 (38.6%)***	22/45 (48.9%)***	22/44 (50.0%)***
	1[c]	1.4	1.9	1.7
Goblet cell hyperplasia All Sites (Females)	3/43 (7.0%)***	19/43 (44.2%)***	24/44 (54.5%)***	28/43 (65.1%)***
	1	1.2	1.3	1.7

[a] Number of lesion-bearing mice/number of mice examined

[b] Asterisks under control column represent linear trend tests; asterisks under dose columns represent pairwise comparisons to controls

*** Significant p-value < 0.001

[c] Indicate severity score based on 1= minimal and 5= marked

Study Conclusions

- Under the conditions of these 2-year studies, there was *clear evidence of carcinogenic activity* of a nondecolorized whole leaf extract of Aloe vera in male and female F344/N rats based upon increased incidences of adenomas and carcinomas of the large intestine.
- There was *no evidence of carcinogenic activity* in male or female $B6C3F_1$ mice exposed to 1%, 2%, or 3% (wt/wt) Aloe vera whole leaf extract in drinking water.
- Exposure to a nondecolorized whole leaf extract of Aloe vera resulted in increased incidences of non-neoplastic lesions of the large intestine in male and female rats and mice, the small intestine of male and female rats, the stomach in male and female rats and female mice, the mesenteric lymph nodes in male and female rats and male mice, and the nose in male mice.